



ISSN: 2231-5101

Characterization of nonpolar and polar solvent extracts from some tea plantation shade tree leaves with special reference to antioxidant and antibacterial activities

Arindam Ghosh, Soumya Majumder, Sumedha Saha and Malay Bhattacharya*

Molecular Biology and Tissue Culture Laboratory, Department of Tea Science, University of North Bengal, Siliguri, West Bengal, India

ABSTRACT

Beneficial properties of shade trees of tea plantations other than their medicinal properties have been extensively studied. This research was initiated to explore properties of some shade trees with special emphasis on their antioxidant and antibacterial properties. Leaves from shade tree like *Dalbergia sissoo* (DS), *Cassia siamea* (CS), *Derris robusta* (DR), *Leucaena leucocephala* (LL), *Acacia lenticularis* (AL) and *Melia azedarach* (MA) were used for the study. Characterization of shade tree leaves by determination of moisture, crude fibre and ash content and tests of non polar – polar solvent extracts for steroid, tannins, cardiac glycosides and coumarin, free radical scavenging, ferric reducing power, NO scavenging activities, quantification of Flavonoids and antibacterial activity were conducted. The average moisture, crude fibre and ash percentage of shade tree plants were found to be 62.95, 11.28 and 1.86 respectively. Methanol, ethanol, acetone and ethyl acetate respectively proved to be the most potent solvent for various phytochemical extractions as it gave positive results for tests like tannin, steroid, cardiac glycosides and coumarin. AL (91.46%), DR (92.69%), LL (94.32%) and MA (93.34%) leaf extracts showed high level of DPPH scavenging activity in their water extracts. In DS (88.11%) and CS (83.23%) maximum DPPH scavenging activity was observed in Diethyl ether and Methanol extracts respectively. Acetone extracts were more active than the water extracts in exhibiting ferric reducing power and NO scavenging activity. Summation of the quantity revealed that DS showed maximum presence of flavonoids and acetone as most potential for isolation of flavonoids. The decreasing order of summative antibacterial activity was recorded in DS, followed by CS, DR, AL, MA and LL. Chloroform showed the highest summative inhibition zone followed by ethanol, ethyl acetate, diethyl ether, acetone, water, hexane, benzene and methanol. Antioxidant and antibacterial potential of shade trees were established.

KEYWORDS: Tea plantation, shade tree, solvents, antioxidant, antibacterial

Received: May 10, 2020
 Accepted: August 02, 2020
 Published: August 30, 2020

*Corresponding Author:
 Malay Bhattacharya
 E-mail: malaytsnbu@gmail.com

INTRODUCTION

Tea, manufactured from the harvested fresh leaves of *Camellia sinensis* (L.) O. Kuntze, is the major plantation crop industry of India. Tea export earns a good amount of foreign exchange. In India tea plantations are large holding covering several hundred hectares of land. Presently, growing tea in small stretch of land has gained momentum, and these small tea growers contribute about half of the total produce. Tea is grown in hills as well in plains. In tea plantations located at lower altitudes and plains, shade trees are inseparable companion to the tea bushes and are considered an indispensable component for the thriving

of the plantation. One or a few species of leguminous plants belonging to the genera *Acacia*, *Albizia*, *Dalbergia*, *Derris*, *Leucaena*, *Cassia* etc. are commonly planted as shade trees in the tea plantations of North East India. Presently, trees like *Melia azedarach*, *Cinnamomum tamala* etc. are also gaining popularity as candidates for fulfilling this role.

The part played by shade trees in the benefit of the plantation is multifaceted. They are primarily planted for the purpose of reducing evapotranspiration which helps with the regulation of temperature in tea gardens at lower altitudes and plains where temperatures rise beyond what is considered optimum.

Copyright: © The authors. This article is open access and licensed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>) which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited. Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made.

In addition to this they serve to increase humidity at the bush level, add humus content to soil and improve nutrient status of the soil by the incorporation of nutrients from shed leaves by subsequently decomposition. They are utilized as well for pest and disease management. Thus, shade trees create a micro climate in the phyllospheric region of tea bushes that facilitates their proper growth and development. Besides influencing and improving tea plantation ecosystem, shade trees are also a potential source of firewood and timber in tea estates.

Previous studies showed that *Dalbergia sissoo* possesses antimicrobial, neural, cardiac, antioxidant, antiparasitic, antidiabetic [1], anti-inflammatory [1&2] and moderate analgesic activities [1&3]. *Melia azedarach* has antifungal and antibacterial properties [4]. *Cassia siamea* has been shown to cure liver problems, loss of appetite from gastrointestinal trouble, insomnia and is antihypertensive [5]. Central American people eat the young leaves, flowers, and young pods of *L. leucocephala* in soups [6-8]. In addition, it is used as contraception, abortifacient to control stomach ache. The leaves have antihelmintic, antibacterial, antiproliferative and antidiabetic activities [8&9]. Even though these beneficial traits associated with shade trees have been studied, but there is no report on comparative medicinal activities of shade tree plants of tea plantations of North Bengal. Consequently, the present research work was designed to explore the comparative phytochemistry of some popularly employed tea plantation shade trees with special emphasis on their antioxidant and antibacterial properties.

MATERIALS AND METHODS

Collection of Plant Materials

Healthy, mature and disease free shade trees were selected from the tea plantation (Latitude 26.71109 and Longitude 88.35053) of University of North Bengal, Siliguri, West Bengal. Leaves from shade tree of *Dalbergia sissoo* Roxb. (DS), *Cassia siamea* Lam. (*Senna siamea* (Lam.) Irwin et Barneby) (CS), *Derris robusta* (Roxb. ex Dc. Benth. (DR), *Leucaena leucocephala* (Lam.) de Wit. (LL), *Acacia lenticularis* Benth. (AL) and *Melia azedarach* L. (MA) were collected early in the morning, packaged separately in ziplock bags and enclosed in an insulated box containing icepacks. Plant materials were identified from digital herbarium facility of Bioinformatics facility of the University.

Determination of Moisture, Crude Fibre, and Ash Content

Moisture loss was determined by drying 10gm of shade tree leaves at 110°C for one hour. The difference in mass was expressed as percentage of moisture present in the samples. Presence of Crude fibres was conducted by acid and alkali digestion. 2 g of shade tree leaves (W_0) was boiled in 200 ml of concentrated sulfuric acid for 30 minutes. The acid extracts were filtered through muslin cloth and washed several times with distilled water until the pH of fibrous residue became neutral. Then the residue was boiled in 200ml of sodium hydroxide

solution for 30 minutes and was filtered again through a muslin cloth. The extract was washed several times with distilled water until the pH of fibrous residue became neutral. The residue was then washed with 25ml of boiling 1.25% H_2SO_4 followed by washing thrice with 50ml distilled water and 25ml of ethyl alcohol. The residue was taken in a crucible and dried in a hot air oven for 2 hours at $130 \pm 2^\circ C$. The crucibles were brought down to room temperature [10]. The residue was cooled down and the masses recorded (W_1). Percentage of crude fibre content was calculated using the formula-

$$\text{Crude fibre (\%)} = (W_1 / W_0) * 100$$

Where weight of the leaves = W_0 and weight of the crude fibre = W_1 .

For determination of ash content, 5gm of shade tree leaves were taken in crucibles and kept in muffle furnace at 600°C for 6 hours. At this temperature water evaporates from the sample and the rest of the contents burn down. Most of the minerals get convert to phosphates, sulphates and other oxides. Percentage of ash content was calculated by the formula-

$$\% \text{Ash} = (\text{weight of residue} / \text{weight of sample taken}) \times 100.$$

Preparation of Extracts for Qualitative and Quantitative Analysis

The leaves were washed thoroughly in running tap water to remove adherent dust particles and finally washed with distilled water. Leaves were then dried with tissue paper and mechanically ground. 3gms of leaves from each sample was soaked with different non-polar to polar solvents (Hexane, Benzene, Chloroform, Di-ethyl ether, Ethyl acetate, Acetone, Ethanol, Methanol, and Water) for 48 hours. The extracts were filtered through Whatman no.1 filter paper. The solvents were dried out at low temperature and finally dissolved in methanol at a concentration of 0.5gm/ml.

Preliminary Phytochemical Group Screening of the Leaf Extracts

The preliminary phytochemical tests were carried out using standard phytochemical methods [11-14]. For test of Steroid 0.5 ml of each leaf extract was evaporated and dissolved in 2ml chloroform. 2 ml of Conc. H_2SO_4 was introduced carefully by pouring down along the inner wall of the test tube. Formation of red color ring confirmed the presence of Steroid. For test of Tannins 0.5 ml of each leaf extract was taken in a clean glass test tube and a few drops of HNO_3 was added to it. The reddish to yellow color change of the solution indicated presence of Tannins. For test of Cardiac glycosides 0.5ml of each leaf extract was evaporated and dissolved in 1ml glacial acetic acid. 1 drop of 10% $FeCl_3$ solution was added followed by 1ml of Conc. H_2SO_4 by pouring carefully down the side of test tube. Appearance of brown color ring at the interface indicated the presence of Cardiac glycosides. For test of coumarin 1ml of each leaf extract was taken in test tube and a few drops of

NaOH solution was added to it. Yellow coloration indicated the presence of coumarin.

Antioxidant Activity Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

Free radical scavenging activity by DPPH assay was conducted following the protocol of Bhattacharya *et al*; 2009 [15]. 0.3ml of each shade tree leaf extract was added to 2 ml of methanol solution of 0.2mM DPPH. The mixture was vortexed vigorously and incubated at room temperature for 30minutes in the dark. Absorbance was measured at 517nm by UV-Vis spectrophotometer. Ascorbic acid ($\mu\text{g/mL}$ methanol) was taken as a standard. DPPH scavenging percentage was calculated as-

$$\text{Free radical scavenging \%} = \left\{ \frac{(\text{Control-Sample})}{\text{Control}} \right\} \times 100.$$

Ferric reducing power assay

Ferric reducing power assay was done by following the protocol of Labar *et al*; 2019 [16]. To 200 μl of each shade tree leaf extract, 500 μl phosphate buffer (pH 7.4) was added. Then, 1% 500 μl $\text{K}_2\text{Fe}(\text{CN})_6$ was added and incubated for 20 minutes at 50°C. After that 10% 500 μl Trichloroacetic acid, 1.5ml distilled water and 0.1% (W/V) 300 μl ferric chloride were added. Then absorbance was measured at 700nm by UV-Vis spectrophotometer.

Nitric Oxide (NO) scavenging activity

To 1ml of each leaf extract, 10Mm 0.5ml sodium nitroprusside was added and kept at 25°C for 150 minutes. 1.2ml Griess reagent was added. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylene diamine was read at 546 nm by UV-Vis spectrophotometer [15].

Quantification of flavonoids

To 250 μl of each leaf extract, 1.25ml double distilled water and 75 μl of 5% NaNO_2 were added and kept at room temperature for 5minutes. Following this, 150 μl of 10% AlCl_3 was added to it and kept for 6minutes at room temperature. After that 500 μl of 1Mm NaOH and 275 μl of double distilled water was added and the solution incubated for 30minutes. Finally absorbance was measured at 510nm by UV-Vis spectrophotometer [16,17].

Antibacterial Activity

The leaf extracts in different solvents were dried and dissolved in 1ml of Dimethyl sulfoxide for antibacterial assay. Overnight grown cultures of *Staphylococcus aureus* (SA), *Bacillus subtilis* (BS), *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KN) were used for the present study. The antibacterial assay of the leaf extracts in different solvents was performed by well diffusion method [16]. Pour plates were made using the media (Mueller Hinton Agar) inoculated with 200 μl inoculum. For the well

diffusion method, 9 wells were made in the dish with a cork-borer and 100 μl of each leaf extract in different solvents were pipetted into the wells. The Petridishes were then incubated overnight at 37°C. Antibacterial activity was discerned by measuring the diameter of the zone of inhibition surrounding the well containing the leaf extracts.

RESULTS AND DISCUSSION

Collection of Plant Materials

Environment plays a crucial role in plant metabolism, so molecules and their quantity vary with the habitat. Keeping this issue in mind, leaves of all the shade trees were collected from a small tea plantation within the campus of University of North Bengal. Leaves from shade trees of *Dalbergia sissoo* (DS), *Cassia siamiae* (CS), *Derris robusta* (DR), *Leucaena leucocephala* (LL), *Acacia lenticularis* (AL) and *Melia azedarach* (MA) were collected in the morning hours to avoid accumulation of photosynthate and loss of water due to transpiration.

Determination of Crude Fibre, Moisture and Ash Percentage

The average percentage of moisture, crude fibre and ash content of the six shade tree plants were found to be 62.95 ± 3.1685 , 11.28 ± 0.0453 and 1.86 ± 0.9835 respectively (Table:1). Except DR and CS all the other shade tree leaves contained moisture above the average. Maximum and minimum moisture percentages were recorded in MA (67.05%) and DR (57.70%) respectively. Except MA and CS all the other shade tree leaves contained crude fibre above the average.

Maximum and minimum crude fibre percentages were recorded in AL (16.65%) and MA (3.55%) respectively. Except DS, CS and MA all the other shade tree leaves contained ash content above the average. Ash content of DS was found to be 1% but comparatively higher percentage of it (19.3-26.1%) was reported in Iraq¹. This could be due to different weather condition.

Maximum and minimum ash content was recorded in LL (3.15%) and MA (0.60%) respectively. Crude fibre of DS was found to be 12.5% but comparatively very higher percentage of it (8.43-12.0%) was reported in Iraq¹. This could be due to less moisture in the environment and more crude fibre content.

Positive correlations were recorded only in between percentage of crude fibre and total ash content (0.6388), but moisture-crude

Table 1: Moisture, crude fibre and ash percentage of shade tree leaves

Shade plants	Moisture %	Crude fibre %	Ash%
AL	63.20 ± 0.5458	16.65 ± 0.4615	2.55 ± 0.1357
DR	57.70 ± 0.5029	14.40 ± 0.3646	2.35 ± 0.1601
DS	64.00 ± 0.6107	12.50 ± 0.1923	1.00 ± 0.0984
CS	61.35 ± 0.5114	9.55 ± 0.3563	1.50 ± 0.1581
LL	64.40 ± 0.7395	11.05 ± 0.3316	3.15 ± 0.2474
MA	67.05 ± 0.3258	3.55 ± 0.1454	0.60 ± 0.0651

fibre (-0.5986) and moisture-ash (-0.3864) content showed negative correlation.

Preliminary Phytochemical Group Screening of the Leaf Extracts

Preliminary phytochemical screening of the leaf extracts for Steroid, Tannins, Cardiac glycosides and Coumarin are depicted in table 2. Methanol, ethanol, acetone and ethyl acetate respectively proved to be the most potent solvent for various phytochemical extractions as it gave positive results for tests like tannin, steroid, cardiac glycosides and coumarin. Preliminary phytochemical groups showed their varied presence in different shade tree species. Tannins in plant

extracts can be pharmacologically useful as astringent [18]. They precipitates proteins in tissue leading to improvement of wound healing [19] and act as antibacterial [20] by inhibiting microbial proliferation by denaturation of enzymes involved in microbial metabolism. Tannin in much quantity was found in DR while DS shows good amount of cardiac glycosides. Cardiac glycosides were reported in LL [18]. Cardiac glycosides are used in the treatment of congestive heart failure and cardiac arrhythmia. Therefore all the plant extracts having cardiac glycosides could be used for this ailment. Steroid was found in good amount in DR, DS and CS. Presence of coumarin was maximum in DR followed by MA, LL, CS, DS and AL. In a previous investigation, LL was tested positive for tannin and reducing sugar [18].

Table 2: Preliminary phytochemical property of shade tree leaf extracts

Solvent	Phytochemical groups	DR	LL	DS	AL	CS	MA
Hexane	Tannin	+	-	-	-	-	-
	Steroid	+	-	+	+	-	-
	Cardiac glycosides	+	+	-	+	+	-
	Coumarin	-	-	-	-	-	-
Benzene	Tannin	++	-	++	+	+	+
	Steroid	+	+	++	+	+	+
	Cardiac glycosides	+	+	+	+	+	+
	Coumarin	+++	+	+	+	-	+
Chloroform	Tannin	++	+	+	++	+	+
	Steroid	+	+	++	++	++	+
	Cardiac glycosides	++	+	+	++	++	+
	Coumarin	+++	+++	-	-	+++	+++
Diethyl ether	Tannin	++	+	+	++	+	+
	Steroid	+	+	++	+	+++	++
	Cardiac glycosides	++	+	++	+	++	+
	Coumarin	+++	+	+	-	+	+++
Ethyl acetate	Tannin	+++	++	++	++	+	+
	Steroid	++	+	+	+	+	++
	Cardiac glycosides	++	++	++	+	++	+
	Coumarin	+++	+++	+++		+++	+++
Acetone	Tannin	+++	++	++	++	++	+
	Steroid	+++	++	++	+	++	+++
	Cardiac glycosides	++	++	+++	++	++	++
	Coumarin	+++	+++	+++	+++	+++	+++
Ethanol	Tannin	+++	++	++	++	+	++
	Steroid	++	+++	++	++	+++	++
	Cardiac glycosides	++	++	+++	+	++	++
	Coumarin	+++	+++	+++	+++	+++	+++
Methanol	Tannin	+++	+++	++	++	++	++
	Steroid	+++	+	+++	++	+++	++
	Cardiac glycosides	++	++	+++	+	++	+
	Coumarin	+++	+++	+++	+	+++	+++
Water	Tannin	+	+	+	+	+	+
	Steroid	+	-	+	-	-	-
	Cardiac glycosides	+	+	+	+	+	+
	Coumarin	+++	+++	+	-	+	+

Antioxidant Activity Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

DPPH activity primarily indicates the ability of plant extracts to scavenge free radicals and this test is considered as an indicator of antioxidant activity of the extract. Non polar to polar solvent extracts of six shade tree leaf extracts showed variable potential of DPPH scavenging activity. Except the leaf extracts of DS and CS all other four shade trees, AL (91.46% or 96.882 μ g AE / mL), DR (92.69%), LL (94.32%) and MA (93.34%) leaf extracts showed high level of DPPH scavenging activity in their water extracts. In an similar research work by DPPH method, good result was recorded in methanol extracts of LL [21]. In DS (88.11%) and CS (83.23%) maximum DPPH scavenging activity was observed in Diethyl ether and Methanol extracts respectively. Lowest level of DPPH scavenging activities were observed in Hexane extracts of all the six shade tree leaves. DPPH scavenging activities were also low in Benzene except for the extracts of DS (55.49%) and DR (71.19%). Chloroform and Diethyl ether extracts of AL, CS and MA showed low activity but extracts of DS, DR and LL showed comparatively high scavenging activity. Ethyl acetate extract of AL (22.88%) showed low DPPH scavenging activity but in DS (61.24%), DR (65.49%), LL (87.33%), CS (48.22%) and MA (52.96%)

the activities of ethyl acetate extracts were moderate or high. Acetone, Ethanol, Methanol and Water extracts of all the shade tree leaves showed moderate, high or very high DPPH scavenging activities. Results depicted in table 3 shows that solvent preference for antioxidant molecules vary from plant to plant. They have a general preference for polar solvents. Water may be considered as ideal solvent for extraction of free radical scavenging molecules from leaves of AL, DR LL and MA, while for DS and CS the ideal solvents are Diethyl ether and Methanol respectively.

Ferric reducing power and NO scavenging activity assay

DPPH scavenging activity alone cannot be considered as the sole test to assess antioxidant activity of plant extracts. So, Ferric reducing power and NO scavenging activity assays were also conducted. Ferric reducing power and NO scavenging activity assay were conducted for Acetone and water extracts only as they responded well in DPPH scavenging activity. The results of Ferric reducing power and NO scavenging activity assay are represented in table 4. In all the shade tree leaves, the acetone extracts were more active than the water extracts in exhibiting ferric reducing power and NO scavenging activity. In acetone extracts of shade tree leaves the FRPA potential increased with increase in concentration for DS, LL, CS and MA while a decrease was observed in case of

Table 3: DPPH scavenging percentage and equivalent Ascorbic acid scavenging activity of shade tree leaf extracts

Solvents	AL		DS		DR		LL		CS		MA	
	*DPPH	**AE	DPPH	AE	DPPH	AE	DPPH	AE	DPPH	AE	DPPH	AE
Hexane	5.19 \pm 0.1069	<1	8.36 \pm 0.1011	2.019	42.57 \pm 0.4072	41.07	10.75 \pm 0.5052	4.747	7.59 \pm 0.1844	1.140	4.85 \pm 0.3772	1
Benzene	12.32 \pm 0.5536	6.539	55.49 \pm 0.9372	55.820	71.19 \pm 0.5169	73.743	14.64 \pm 0.2762	9.188	8.8 \pm 0.1527	2.521	11.21 \pm 0.1637	5.272
Chloroform	28.64 \pm 0.3252	25.170	64.46 \pm 0.4563	66.060	74.89 \pm 0.4021	74.966	91.54 \pm 0.4508	96.973	25.84 \pm 0.3655	21.973	10.9 \pm 0.3914	4.918
Diethyl ether	42.51 \pm 0.4257	41.003	88.11 \pm 0.6058	93.058	66.83 \pm 0.4250	68.765	89.21 \pm 0.3593	94.313	13.92 \pm 0.1950	8.366	25.52 \pm 0.2369	21.608
Ethyl acetate	22.88 \pm 0.3027	18.594	61.24 \pm 0.2523	62.384	65.49 \pm 0.5109	67.236	87.33 \pm 0.8311	92.167	48.22 \pm 0.4968	47.521	52.96 \pm 0.3555	52.932
Acetone	86.7 \pm 0.4119	91.448	55.12 \pm 0.6023	55.398	68.62 \pm 0.4618	70.809	80.63 \pm 0.3153	84.519	76.2 \pm 0.3910	79.462	80.31 \pm 0.4554	84.154
Ethanol	74.01 \pm 0.9003	76.962	75.32 \pm 0.4063	78.457	57.58 \pm 0.5056	58.206	81.01 \pm 0.2909	84.953	72.19 \pm 0.2400	74.884	76.93 \pm 0.1715	80.295
Methanol	90.98 \pm 0.3300	96.334	81.34 \pm 0.4576	85.329	74.85 \pm 0.5488	77.921	89.36 \pm 0.1400	82.769	83.23 \pm 0.6404	87.487	82.39 \pm 0.2474	86.528
Water	91.46 \pm 0.5225	96.882	86.32 \pm 0.2969	91.014	92.69 \pm 0.2793	98.286	94.32 \pm 0.2650	100.147	54.97 \pm 0.3050	55.227	93.34 \pm 0.2797	99.028

*DPPH (% scavenging) and **Ascorbic acid equivalent (μ g AE / ml) was calculated from the standard curve, $y=0.876x+6.591$, $R^2 = 0.993$

Table 4: Ferric reduction power assay (FRPA) and NO scavenging activity of solvent extracts

Assay (0.4mg/ μ l)	Solvent	Sample (μ l)	AL	DS	DR	LL	CS	MA
FRPA	Acetone	100	3.6918 \pm 0.0394	3.6708 \pm 0.0139	4.2777 \pm 0.0272	4.4078 \pm 0.0862	3.7656 \pm 0.1608	3.5960 \pm 0.1447
		200	3.5716 \pm 0.0248	4.2783 \pm 0.0279	4.1864 \pm 0.1037	5.3983 \pm 0.2489	3.8924 \pm 0.0674	3.6356 \pm 0.0226
	Water	100	3.4003 \pm 0.0286	3.4449 \pm 0.0734	4.1684 \pm 0.1053	3.8934 \pm 0.0421	3.2953 \pm 0.0253	3.4839 \pm 0.0166
		200	3.5040 \pm 0.0448	3.2412 \pm 0.0995	3.3765 \pm 0.1013	3.7269 \pm 0.0734	3.4641 \pm 0.0114	3.5965 \pm 0.0227
NO	Acetone	100	2.7239 \pm 0.0160	3.6175 \pm 0.0663	2.5277 \pm 0.0448	2.4831 \pm 0.0422	2.5391 \pm 0.1210	2.5058 \pm 0.1131
		200	3.2012 \pm 0.1164	3.7775 \pm 0.0321	3.1062 \pm 0.0575	3.0242 \pm 0.5299	2.9645 \pm 0.3542	2.9129 \pm 0.0509
	Water	100	1.8264 \pm 0.1331	1.8115 \pm 0.1096	2.0017 \pm 0.0653	1.9244 \pm 0.0319	1.9685 \pm 0.1016	1.8982 \pm 0.0465
		200	1.8031 \pm 0.1187	1.8850 \pm 0.1112	1.9133 \pm 0.0507	1.8987 \pm 0.1118	1.8663 \pm 0.1680	1.8957 \pm 0.1651

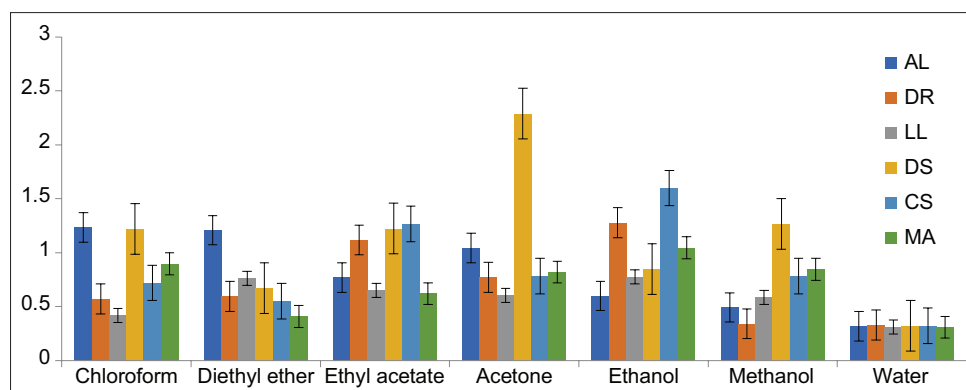


Figure 1: Quantity of flavonoids as quercetin equivalent detected in solvent extracts

AL and DR. In water extracts of shade tree leaves the FRPA potential increased with increase in concentration for AL, CS and MA but decreased in DS, DR and LL. Among the shade tree leaves experimented on, maximum FRPA was observed in acetone extracts of LL at concentration of (0.4mg/μl) while the lowest activity was noted in water extract of DS at the same concentration. It was observed that the shade tree leaf extracts with acetone showed better NO scavenging activity than water extracts. In acetone extracts of all the shade tree leaves the NO scavenging activity increased with increase in concentration, but in water extracts of these shade tree leaves the NO scavenging activity increased with increase in concentration only in DS and decreased in AL, DR, LL, CS and MA. Among the shade tree leaves experimented on, maximum NO scavenging activity was observed in acetone extracts of DS at concentration of (0.4mg/μl) while the lowest activity was recorded in water extract of AL at the same concentration.

Quantification of flavonoids

Flavonoids are considered as important antioxidant molecules that appear to possess a variety of mechanisms of action including free radical scavenging [22]. The presences of flavonoids in the extracts are tested for their antioxidant activity. Flavonoids are anti-allergic, anti-inflammatory, antimicrobial and anti-cancer [23]. Quantification of Flavonoids was conducted in Chloroform, Diethyl ether, Ethyl acetate, Acetone, Ethanol, Methanol and Water extracts of shade tree plant leaves. Determination of total flavonoid content expressed as mg Quercetin equivalent (QE)/g in fresh leaves of shade tree extracted by different solvents (chloroform-water). The quantities of flavonoids detected were converted to quercetin equivalent and are represented in figure 1. The shade tree and the solvents responded with much variability. Maximum amount of flavonoids were recorded in acetone extracts of DS (2.288 QE)/g) while lowest in water extract of MA (0.307QE)/g). Summation of the quantity of flavonoids in all the solvents revealed that DS (1.119 QE)/g) showed maximum while LL (0.585 QE)/g) showed minimum quantity of flavonoids. Similarly, summation of the quantity of flavonoids in all the shade trees revealed, Acetone (1.05 QE)/g) showed maximum while water (0.317 QE)/g) showed minimum quantity of flavonoids.

Solvent	Organism	AL	DR	DS	CS	LL	MA
Hexane	EC	0.3	0.3	0	0.6	0	0
	KP	0.45	0.2	0.35	0.2	0.1	0
	BS	0	0	0.05	0	0.15	0
	SA	0.3	0.17	0.3	0	0	0.2
Benzene	EC	0.4	0	0.3	0	0	0
	KP	0.45	0.4	0.5	0	0.2	0.1
	BS	0.1	0	0	0	0.1	0.45
	SA	0	0.2	0.35	0	0	0
Chloroform	EC	0.3	0	0.45	0	0	0.3
	KP	0.5	0	0.5	0	0	0.3
	BS	0	1.6	0	1.5	0	0
	SA	0	0.17	1.35	0	0	0.2
Diethyl ether	EC	0.4	0	0.4	0.45	0	0.4
	KP	0	0.3	0.5	0	0	0.1
	BS	0	0.5	0.2	0	0.2	0.3
	SA	0	0.2	0.3	0	0	0.1
Ethyl acetate	EC	0.6	0.25	0.6	0	0	0
	KP	0	0	0.4	0	0	0.3
	BS	0	0.6	0	1.3	0	0.2
	SA	0.3	0.25	0.6	0	0.4	0
Acetone	EC	0.4	0.2	0.8	0	0	0.3
	KP	0.4	0	0.1	0	0	0
	BS	0	0	0	0.75	0	0.35
	SA	0.2	0.2	0.55	0	0	0
Ethanol	EC	0.4	0.5	0.9	0	0	0.3
	KP	0	0.35	0.4	0	0	0.5
	BS	0	0	0	1.8	0	0.3
	SA	0	0.17	0.4	0.2	0.3	0
Methanol	EC	0.3	0	0.5	0	0	0.35
	KP	0	0	0.7	0.3	0	0
	BS	0	0	0	0	0	0.2
	SA	0	0.25	0.35	0.35	0.1	0.1
Water	EC	0.3	0.3	0.2	0.4	0	0
	KP	0.3	0	0.5	0	0	0.55
	BS	0.35	0	0	0	0	0
	SA	0	0.5	0.2	0	0	0.2

Figure 2: Heatmap representing inhibition zones produced by non polar-polar solvent extracts of shade tree leaves

Antibacterial Activity

The extracts obtained from the shade tree leaves using nine non polar to polar solvents exhibited varied degree of inhibition zones against two gram positive and two gram negative bacteria. The result of bacterial growth inhibition experiment has been represented by heatmap (Figure 2). The

summation of zones of inhibition produced by the extracts against the organisms showed that there is no strict preference against gram positive and negative bacteria. The inhibition zone produced by EC (12.2cm), BS (11cm), KP (9.95cm) and SA (9.475cm) were close in magnitude. Maximum summative antibacterial activity was recorded in DS (25.35cm) followed by CS (16.7cm), DR (15.775cm), AL (12.1cm), MA (12.0cm) and LL (2.95cm) with the least. The non-polar to polar solvents responded unequally towards extraction of antibacterial molecules from the shade tree leaves and the results were exhibited in the inhibition zones they produced against bacterial growth. Chloroform (7.175cm) showed the highest summative inhibition zone followed by ethanol (6.525cm), ethyl acetate (5.8cm), diethyl ether (4.35cm), acetone (4.25cm), water (3.8cm), hexane (3.675cm), benzene (3.55cm) and methanol (3.5cm).

CONCLUSION

Tea being the most important plantation based industry of India forms the backbone of economy in some regions of the country. Trees planted to provide shade to tea bushes may have potential medicinal activities that may be exploited. Shade trees are rich in steroid, tannins, cardiac glycosides and coumarin. High level of antioxidants and antibacterial activities were detected in some of the shade trees. Shade trees have preferences for solvents as all the solvent extracts were not found to be equally potential for all the tests. Solvents like water (AL, DR LL and MA), diethyl ether (DS) and methanol (CS) for extraction of free radical scavenging molecules, acetone for flavonoids and chloroform for anti bacterial molecules were found to be useful. Thus we can clearly infer that selection of ideal solvent is essential for isolation or *in vitro* studies. So, the collective information of this research work could promote future research to utilize shade trees of tea plantations in an efficient manner. This initiative may further glorify tea industry by promoting more employment and income opportunities.

ACKNOWLEDGEMENT

The authors would like to acknowledge the scientific team of Digital Herbarium Facility of Bioinformatics Facility, University of North Bengal for identifying the plant specimens.

AUTHOR CONTRIBUTIONS

AG, SM and SS carried out the collection, experiments and data analysis. AG wrote the first draft of the manuscript. MB conceived the idea of the study, participated in its design and coordinated to draft the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Al-Snafi, AE. Chemical constituents and pharmacological effects of *Dalbergia sissoo*-A review. IOSR Journal of Pharmacy. 2017; 7(2), 59-71.
2. Hajare SW, Chandra S, Sharma J, Tandan SK, Lal J, & Telang AG. Anti-inflammatory activity of *Dalbergia sissoo* leaves. Fitoterapia. 2001; 72(2), 131-139.
3. Hajare SW, Chandra S, Tandan SK, Sarma J, Lal J, & Telang AG. Analgesic and antipyretic activities of *Dalbergia sissoo* leaves. Indian Journal of Pharmacology. 2000. 32(6), 357-360.
4. Al-Rubae A.Y. The potential uses of *Melia azedarach* L. as pesticidal and medicinal plant, review. American-Eurasian Journal of Sustainable Agriculture. 2009; 3(2), 185-194.
5. Veerachari U. & Bopaiah A. K. Preliminary phytochemical evaluation of the leaf extract of five *Cassia species*. Journal of Chemical and Pharmaceutical research, 2011; 3(5), 574-583.
6. Hegarty MP, Schinckel PG, Court RD. Reaction of sheep to the consumption of *Leucaena glauca* and to its toxic principle mimosine. Australian Journal of Agricultural Research. 1964; 15:53-67.
7. Rushkin FR. ed. *Leucaena*: promising forage and tree crops for the tropics. 2nd ed. National Research Council. Washington, DC: National Academy Press; 1984.
8. Zayed MZ & Samling, B. Phytochemical constituents of the leaves of *Leucaena leucocephala* from Malaysia. International Journal of Pharmacy and Pharmaceutical Science. 2016; 8(12), 174-179.
9. Meena Devi VN, Ariharan VN, Nagendra Prasad P. Nutritive value and potential uses of *Leucaena leucocephala* as biofuel—a mini review. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013; 4:515–21..
10. Śmiechowska M & Dmowski P. Crude fibre as a parameter in the quality evaluation of tea. Food chemistry. 2006; 94(3), 366-368.
11. Brain KR & Turner TD. The Practical Evaluation of Phytopharmaceuticals. Bristol: Wright-Scientific; 1975.
12. Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P, Padmanabhan N, et al. Phytochemicals investigation on a tropical plant, *Syzygium cumini* from Kattupalayam, Erode district, Tamil Nadu, South India. Pakistan Journal of Nutrition. 2009; 8(1):83-85.
13. Ngbede J, Yakubu RA, Nyam DA. Phytochemical screening for active compounds in *Canarium schweinfurthii* (Atile) leaves from Jos North, Plateau State, Nigeria. Research Journal of Biological Sciences. 2008; 3(9):1076-1078.
14. Labar R, Sen A. & Bhattacharya M. Effect of solvents on qualitative and quantitative phytochemical constituent profiles of fresh leaves of TV26. NBU Journal of Plant Science. 2019; 11, 115-123.
15. Bhattacharya M, Mandal P, & Sen A. In vitro detection of antioxidants in different solvent fractions of Ginger (*Zingiber officinale* Rosc.). Indian Journal of Plant Physiology. 2009; 14(1), 23-27.
16. Labar R, Sarkar I, Sen A and Bhattacharya M. Effect of solvent with varying polarities on phytochemical extraction from mature tea leaves and its evaluation using biochemical, antimicrobial and in-silico approaches. International Research Journal of Pharmacy. 2019; 10(8):59-67.
17. Zou Y, Lu Y, Wei D. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. in vitro. Journal of Agricultural and Food Chemistry. 2004; 52(16):5032-5039.
18. Awe FA, Giwa-Ajeniya AO, Akinyemi AA, Ezeri GNO. Phytochemical analysis of *Acalypha wilkesiana*, *Leucaena leucocephala*, *Peperomia pellucida* and *Sena alata* leaves. The International Journal of Engineering and Sciences. 2013; 2:41-4..
19. Tyler, V.E., Braddy, L.R. and Roberts, J.E. (1998). A textbook of pharmacology Lea and Pernoager, Philadelphia Pg 85-90.
20. Awosika F. Local Medicinal Plants and Health of Consumers Clinical Pharmacology. Herbal Medicine. 1991, 9: 28-29.
21. Zarina Z, Ghazali CMR and Sam ST. Characterization analysis for leaves of *Leucaena leucocephala* by using phytochemical screening assay. AIP Conference Proceedings 2017; vol 1885, 020260; <https://doi.org/10.1063/1.5002454>
22. Bhattacharya M. & Chakraborty S. Free Radicals and Naturally Occurring Antioxidants. Journal of Pharmacognosy and Phytochemistry. 2015; 3:3,1-7.
23. Balch JF. & Balch PA. Prescription for nutritional healing. New York: A very. Penguin Putnam Inc. *Vernonia amygdalina* used by wild chimpanzees. Tetrahedron. 2000. 48, 625-630.